REMARKS

In the current application, Claims 1, 3-5, 7-19, and 32-33 have been previously canceled. Claims 2, 6, and 31 remain under consideration. Applicants acknowledge that Claim 34 was pending, thank the Examiner for pointing out the discrepancy in the record, and cancel Claim 34 as drawn to non-elected subject matter, reserving the right to file a divisional application to all non-elected subject matter.

Objection or Rejection Withdrawn in Light of Applicants' Previous Submission

Specification:

Applicants note that, on page 2 of the Final Office Action, the objection to the disclosure is withdrawn in light of the previously entered biological deposit information.

Patentability Under 35 USC § 112, second paragraph

Applicants note that on page 6 of the Final Office Action, the rejection of Claim 6 under 35 USC § 112, second paragraph, as being indefinite, is withdrawn based upon the previous amendment,.

Patentability Under 35 USC § 102

Applicants note that on page 7 of the Final Office Action, the rejection of Claim 6 as being anticipated by Daniel *et al.* is withdrawn based upon the previous amendment.

Double Patenting

Applicants acknowledge that the rejection of Claim 6 as being unpatentable over Claims 1-10 and 13-16 of U.S. Patent No. 5,686,276 in view of Daniel *et al.* (1992), in light of Daniel *et al.* (1995), has been withdrawn, based upon the previously entered amendment.

Pending Rejections

Double Patenting (judicially created doctrine of obviousness-type)

U.S. Pat. No. 6,025,184:

Claims 2, 6, and 31 stand rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over Claims 1-4 of U.S. Patent No. 6,025,184. Applicants traverse the rejection for reasons of obviousness.

US 6,025,184 (Ser. No. 08/966,794) is a division of application Ser. No. 08/440,293, filed May 12, 1995, now U.S. Pat. No. 5,686,276, issued Nov. 11, 1997.

A requirement for restriction to one of the following patentably distinct inventions in Ser. No. 08/440,293 was imposed under 35 U.S.C. §121:

I. Claims 1-13, drawn to a process for the bioconversion of a carbon substrate to 1,3-propanediol.

II. Claim 14, drawn to products *per se*.

III. Claims 15-19, drawn to cosmids and transformed microorganisms.

The Applicants elected Group III to prosecute in Ser. No. 08/966,794 and U.S. Patent No. 6,025,184 subsequently issued with the following composition claims:

1. A cosmid contained in ATCC 69789 comprising a DNA fragment of about 35 kb isolated from *Klebsiella pneumoniae* wherein 1) the DNA fragment encodes an active glycerol dehydratase enzyme and 2) digestion of the cosmid results in a restriction digest pattern as shown in FIG. 1, columns 1 and 2.

2. A host bacterium transformed with the cosmid of claim 1.

3. The host bacterium of Claim 2 which is deposited with the American Type Culture Collection and having accession number ATCC 69789.

4. A host bacterium comprising the cosmid of Claim 1, wherein at least one DNA fragment of said cosmid encodes 1,3-propanediol oxidoreductase, and wherein said host converts a carbon source, other than glycerol or dihydroxyacetone, to 1,3-propanediol.

Applicants respectfully disagree with the Examiner that Claims 1-4 of US Patent No: 6025184 can be used support a finding of obviousness-type double-patenting. The claims currently under examination likewise claim priority back to US Application No. 08/440,293. This application is a division of Application Serial No. 08/439,404, filed 22 May 1997, which was the National Stage of International Application, filed in the PCT as PCT/96/06705 on 10 May 1996 designating the United States of America, which in turn was a continuation-in-part application of US Application No. 08/440,293, filed 12 May 1995, issued 11 November 1997 as US Pat. No. 5,686,276. A restriction requirement issued in the instant case as well, and the pending claims belong to Invention I, drawn to bioprocesses and materials to produce 1,3-propanediol using glycerol or dihydroxyacetone as the substrate.

Additionally with regard to Claim 31, while the natural regulatory elements from one enteric bacterium (for example, the *K. pneumoniae* DNA within the cosmid pTC1) may function in another enteric bacterium (for example, *E. coli*), before Applicants' invention there existed the difficulty of expressing a multiple subunit, prokaryotic enzyme in eucaryotic organisms. Daniel and Gottschalk (FEMS Microbiol. Lett. (1992) 100:281-286), for example, disclose the anaerobic production

of 1,3-propanediol from glycerol using an *E. coli* transformed with the *Citrobacter freundii* genes encoding the *dha* regulon. The genes (*e.g.*, genes encoding glycerol dehydratase) from one enteric bacterium (*Citrobacter*) were transformed into and shown to function within a different enteric bacterium (*Escherichia*). However, Daniel and Gottschalk (1992) clearly would not have suggested to a person skilled in the art the use of a recombinant eucaryotic microorganism for expression of a glycerol dehydratase enzyme.

In light of the above discussion, Applicants respectfully request reconsideration of the claims as amended, withdrawal of the rejection, and prompt allowance of the application.

U.S. Patent No. 5,821,092:

Claims 2 and 6 stand rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over Claims 1-10 of U.S. Patent No. 5,821,092. This patent is a division of application Ser. No. 08/440,377 (filed May 12, 1995, now U.S. Pat. No. 5,633,362).

U.S. Patent No. 5,821,092 focuses on the presence of a diol dehydratase enzyme in the biological material and process and claims:

1. A process for the bioconversion of a carbon substrate for diol dehydratase enzyme to the corresponding product comprising the steps of:

(i) transforming a microbial host with genes encoding an enzymatically active bacterial diol dehydratase enzyme, the genes derived from

(1) a cosmid, the cosmid comprising a DNA fragment of about 35 kb isolated from Klebsiella pneumoniae and contained within transformed E. coli deposited with the American Type Culture Collection under accession number ATCC 69790; or from

(2) enzymatically active diol dehydratase genes isolated from the group consisting of members of the species Klebsiella sp., Clostridia sp., Salmonella sp. and Citrobacter sp, one subunit of the genes and having at least a 95% identity to the nucleic acid sequence of SEQ ID NO:1;

(ii) contacting the transformed microbial host with the carbon substrate in a suitable medium; and

(iii) recovering the corresponding product from the suitable medium.

2. The process of claim 1 wherein the carbon substrate is selected from the group consisting of ethylene glycol, 1,2-propanediol, glycerol and 2,3-butanediol.

3. The process of claim 2 wherein the carbon substrate is glycerol.

4. The process of claim 3 wherein the glycerol is converted to 1,3-propanediol.

5. The process of claim 1 wherein the microbial host is selected from the group consisting of members of the genera Eschericia, Bacillus, Klebsiella, Citrobacter, Saccharomyces, Clostridium and Pichia.

6. The process of claim 5 wherein the microbial host is selected from the group consisting of members

of species E. coli, Bacillus subtilis, Bacillus licheniformis and Pichia pastoris.

7. The process of claim 6 wherein the microbial host is E. coli.

8. The process of claim 1 wherein (a) the transformed microbial host is recombinant E. coli DH5.alpha. containing a gene encoding an enzymatically active diol dehydratase enzyme, the gene comprising the DNA sequence of SEQ ID NO. 1; (b) the carbon substrate is glycerol; and (c) the product recovered in step (iii) is 1,3-propanediol.

9. A process for the bioconversion of glycerol to 1,3-propanediol comprising the steps of:

(i) transforming a microbial host selected from the group consisting of the genera Eschericia, Bacillus, Klebsiella, Citrobacter, Saccharomyces, Clostridium and Pichia with genes encoding an enzymatically active bacterial diol dehydratase enzyme, the genes derived from a cosmid, the cosmid comprising a DNA fragment of about 35 kb isolated from Klebsiella pneumoniae, the cosmid contained within transformed E. coli deposited with the American Type Culture Collection under accession number ATCC 69790;

(ii) contacting the transformed microbial host with carbon substrate in a suitable medium; and

(iii) recovering 1,3-propanediol from a suitable medium.

10. The process of claims 8, 1 or 9 wherein the transformed microbial host further contains an alcohol dehydrogenase.

Applicants respectfully disagree with the Examiner that Claims 1-10 of US Patent No: 5,821,092 can be used support a finding of obviousness-type double-patenting. Applicants have amended Claim 2 to claim a process using a microorganism having "at least one exogenous gene from *Klebsiella* or *Citrobacter* expressing a glycerol dehydratase enzyme." The Claim as amended requires an exogenous gene expressing glycerol dehydratase, not diol dehydratase, the subject of the '092 patent.

In light of the above discussion, Applicants respectfully request reconsideration of the claims as amended, withdrawal of the rejection, and prompt allowance of the application.

U.S. Patent No. 5,633,362:

Claims 2, 6, and 31 stand rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over Claims 1-10 of U.S. Patent No. 5,633,362.

U.S. Patent No. 5,633,362, focusing on the presence of a dial dehydratase enzyme in the bioprocess and biomaterials, claims:

1. A cosmid comprising a DNA fragment of about 35 kb isolated from Klebsiella pneumoniae wherein said fragment encodes an active diol dehydratase enzyme having the restriction digest in FIG. 5, columns numbered 4, said cosmid contained within a transformed E. coli deposited with the American Type Culture Collection under accession number ATCC 69790.

2. A transformed microorganism comprising a host microorganism and the cosmid of claim 1.

3. The transformed microorganism of claim 2 wherein the host microorganism is E. coli, and which is deposited with the American Type Culture Collection as accession number ATCC 69790.

4. The cosmid of claim 1 which when transformed into bacteria causes metabolism of glycerol to 1,3-propanediol.

5. A transformed microorganism comprising a host microorganism and a DNA fragment of the cosmid of claim 1, said fragment encoding an active functional protein.

6. A DNA fragment comprising a gene encoding a diol dehydratase enzyme, said gene encompassed by the cosmid of claim 1.

7. A isolated gene encoding an active diol dehydratase enzyme comprising a contiguous sequence which consists of SEQ ID NO: 1.

8. A isolated gene encoding an active alcohol dehydrogenase comprising a contiguous sequence which consists of SEQ ID NO: 2.

9. A transformed microorganism comprising a host microorganism and the heterologous gene of claim 7 or claim 8.

10. A transformed microorganism comprising E. coli DH5.alpha. and the DNA sequence of claim 7 or claim 8.

Applicants respectfully disagree with the Examiner that Claims 1-10 of US Patent No: 5,633,362 can be used support a finding of obviousness-type double-patenting. Applicants have amended Claim 2 to claim a bioprocess using a microorganism having "at least one exogenous gene from *Klebsiella* or *Citrobacter* expressing a glycerol dehydratase enzyme." The Claim as amended requires an exogenous gene expressing glycerol dehydratase, not diol dehydratase which is the subject of the '362 patent.

In light of the above discussion, Applicants respectfully request reconsideration of the claims as amended, withdrawal of the rejection, and prompt allowance of the application.

U.S. Patent No. 6,013,494:

Claims 2, 6, and 31 stand rejected under the judicially created doctrine of obvious-type double patenting as being unpatentable over Claims 1-13 of U.S. Patent No. 6,013,494 (This application claims the benefit of U.S. Provisional Application No. 60/030,601, filed Nov. 13, 1996 (Attorney Docket No: CR-9982)).

U.S. Patent No. 6,013,494 claims:

1. A method for the production of 1,3-propanediol from a recombinant microorganism comprising:

(i) transforming a suitable host microorganism with one or more transformation cassettes each of which comprises at least one of

(a) a gene encoding a glycerol-3-phosphate dehydrogenase activity;

(b) a gene encoding a glycerol-3-phosphatase activity;

(c) genes encoding a dehydratase activity; and

(d) a gene encoding 1,3-propanediol oxidoreductase activity,

wherein all of the genes of (a)-(d) are introduced into the host microorganism;

(ii) culturing the transformed host microorganism under suitable conditions in the presence of at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, or a one-carbon substrate whereby 1,3-propanediol is produced; and

(iii) recovering the 1,3-propanediol.

2. The method of claim 1 wherein the suitable host microorganism is selected from the group consisting of bacteria, yeast, and filametous fungi.

3. The method of claim 2 wherein the suitable host microorganism is selected from the group of genera consisting of Citrobacter, Enterobacter, Clostridium, Klebsiella, Aerobacter, Lactobacillus, Aspergillus, Saccharomyces, Schizosaccharomyces, Zygosaccharomyces, Pichia, Kluyveromyces, Candida, Hansenula, Debaryomyces, Mucor, Torulopsis, Methylobacter, Escherichia, Salmonella, Bacillus, Streptomyces and Pseudomonas.

4. The method of claim 3 wherein the suitable host microorganism is selected from the group consisting of E. coli, Klebsiella spp., and Saccharomyces spp.

5. The method of claim 1 wherein the transformed host microorganism is a Klebsiella spp. transformed with a transformation cassette comprising the genes GPD1 and GPP2.

6. The method claim 1 wherein the carbon source is glucose.

7. The method of claim 1 wherein the gene encoding a glycerol-3-phosphate dehydrogenase activity is selected from the group consisting of (a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:11, in SEQ ID NO:12, and in SEQ ID NO:13, or an enzymatically active fragment thereof;

(b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1.times.SSC, 0.1% SDS at 65.degree. C.; and

(c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

8. The method of claim 1 wherein the gene encoding a glycerol-3-phosphatase activity is selected from the group consisting of

(a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:33 and in SEQ ID NO:17, or an enzymatically active fragment thereof;

(b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1.times.SSC, 0.1% SDS at 65.degree. C.; and

(c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

9. The method of claim 1 wherein the gene encoding a glycerol-3-phosphatase activity is a glycerol kinase gene selected from the group consisting of

(a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:18, or an enzymatically active fragment thereof;

(b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1.times.SSC, 0.1% SDS at 65.degree. C.; and(c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

10. The method of claim 1 wherein the genes encoding a dehydratase activity comprise dhaB1, dhaB2 and dhB3, and are selected from the group consisting of

(a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, or an enzymatically active fragment thereof;

(b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1.times.SSC, 0.1% SDS at 65.degree. C.; and(c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

11. The method of claim 1 wherein the gene encoding a 1,3-propanediol oxidoreductase activity selected from the group consisting of

(a) an isolated nucleic acid molecule encoding the amino acid sequence set forth in SEQ ID NO:37, or an enzymatically active fragment thereof;

(b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1.times.SSC, 0.1% SDS at 65.degree. C.; and

(c) an isolated nucleic acid molecule that is completely complementary to (a) or (b).

12. A method for the production of 1,3-propanediol from a recombinant microorganism comprising:

(i) culturing, under suitable conditions in the presence of at least one carbon source selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, or a one-carbon substrate, a transformed host microorganism comprising

(a) a gene encoding a glycerol-3-phosphate dehydrogenase activity;

(b)a gene encoding a glycerol-3-phosphatase activity;

(c)genes encoding a dehydratase activity; and

(d) a gene encoding 1,3-propanediol oxidoreductase activity,

wherein all of the genes (a)-(d) are exogenous to the host microorganism, whereby 1,3-propanediol is produced; and

(ii) recovering the 1,3-propanediol.

13. A host cell transformed with a group of genes comprising:

(1) a gene encoding a glycerol-3-phosphate dehydrogenase enzyme corresponding to the amino acid sequence given in SEQ ID NO:11;

(2) a gene encoding a glycerol-3-phosphatase enzyme corresponding to the amino acid sequence given in SEQ ID NO:17;

(3) a gene encoding the a subunit of the glycerol dehydratase enzyme corresponding to the amino acid sequence given in SEQ ID NO:34;

(4) a gene encoding the .beta. subunit of the glycerol dehydratase enzyme corresponding to the amino acid sequence given in SEQ ID NO:35;

(5) a gene encoding the .gamma. subunit of the glycerol dehydratase enzyme corresponding to the

amino acid sequence given in SEQ ID NO:36; and

(6) a gene encoding the 1,3-propanediol oxidoreductase enzyme corresponding to the amino acid sequence given in SEQ ID NO:37,

whereby the transformed host cell produces 1,3-propanediol on at least one substrate selected from the group consisting of monosaccharides, oligosaccharides, and polysaccharides or from a one-carbon substrate.

In light of the above discussion, Applicants respectfully request reconsideration of the claims as amended, withdrawal of the rejection, and prompt allowance of the application.

Patentability under 35 USC § 112, first paragraph

Claims 2, 6, and 31 remain rejected under 35 USC § 112, first paragraph, for lacking enablement of the claimed inventions. The Examiner asserts that while Applicants have enabled a process for generating 1,3-propanediol with microorganisms transformed with the *Klebsiella dhaB* gene, the specification does not reasonably provide enablement for production of 1,3-propanediol with any diol dehydratase gene from any other organism. The Examiner finds that the scope of the claims is much broader than the enablement provided by the specification and that undue experimentation would be required to obtain other dehydratase genes with which to practice the claimed invention.

Applicants have amended process Claim 2 and composition Claim 31 to recite that the microorganism has "at least one exogenous gene from *Klebsiella* or *Citrobacter* expressing a glycerol dehydratase enzyme"... At the time the application was filed it would not have required undue experimentation to apply the teachings of the specification, in combination with its detailed examples, to isolate other genes encoding a glycerol dehydratase commensurate with the instant invention.

The specification discloses the use of fungal microorganisms as hosts for a gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter*. The glycerol dehydratase gene from *Klebsiella* was provided in the specification, and *dhaB* from *Citrobacter* was used in the present application to identify *dhaB* in *K. pneumoniae*. The working examples refer to a glycerol dehydratase gene from *Klebsiella*, and the Detailed Description teaches methods for isolating cosmids and genes (See pages 9-11). The specification also refers to *Citrobacter, Clostridium,* and *Salmonella*, which were known to contain dehydratase activity (See page 11, line 8).

Claim 31 as amended describes a recombinant eucaryotic microorganism that expresses a glycerol dehydratase enzyme. It was unknown, until disclosed by Applicants, that dehydratase genes can be made to function in microorganism species

more distantly related than enteric bacteria. Applicants have demonstrated that these genes (*e.g.*, genes from *Klebsiella or Citrobacter* encoding glycerol dehydratase enzyme) are fully functional in *Streptomyces*, *Bacillus*, and *Pseudomonas* species, and also in eucaryotic species such as *Pichia*, *Saccharomyces*, and *Aspergillus*. Example 7 details the construction of a recombinant *Pichia*; Examples 9-11 teach the construction of recombinant *Saccharomyces*; and Examples 22-23 detail a recombinant *Aspergillus*. Each of these recombinants expresses an active dehydratase enzyme. There is enabling teaching in the working examples to support the scope of the claims as amended with respect to the use of fungal host cells.

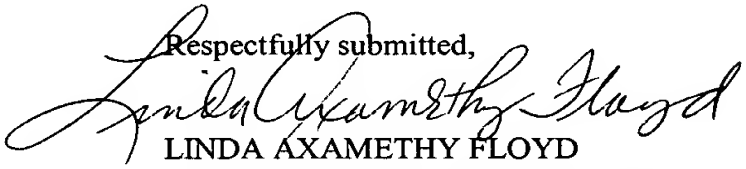
Furthermore, within the specification, the Applicants have specifically identified the multiple glycerol dehydratase genes: "Referring to SEQ ID NO:1, --- the open reading frame *dhaB1* encoding the alpha subunit glycerol dehydratase is found at bases 7044-8711; the open reading frame *dhaB2* encoding the beta subunit glycerol dehydratase is found at bases 8724-9308; the open reading frame *dhaB3* encoding the gamma subunit glycerol dehydratase is found at bases 9311-9736 ---".

Having first correctly identified the *alpha, beta,* and *gamma* subunits of glycerol dehydratase, the Applicants further coordinately expressed each of the subunits in yeasts (*Pichia*, Example 7; *Saccharomyces*, Example 9) and filamentous fungi (*Aspergillus*, Example 22) without the advantage of a natural, single bacterial promoter driving the expression of multiple polypeptides.

In light of the above discussion, Applicants respectfully request reconsideration of the claims as amended, withdrawal of the rejection for lack of enablement, and prompt allowance of the application.

Should any matter remain unresolved, please contact the undersigned as indicated.

Respectfully submitted,



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